Revised Test Plan for

Sunset Yellow

CAS No. 2783-94-0

Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

The International Association of Color Manufacturers/HPV Committee

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.

Table of Contents

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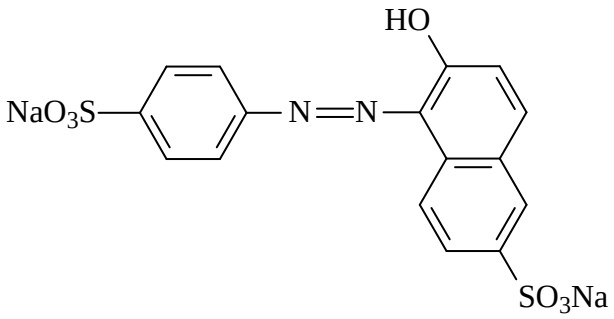
1 IDENTITY OF SUBSTANCES



Sunset Yellow

CAS No. 2783-94-0

Synonyms:
FD&C Yellow 6
C.I. Food Yellow 3

2 CATEGORY ANALYSIS

2.1 INTRODUCTION

The International Association of Color Manufacturers (IACM) has volunteered to participate in the EPA's Chemical "Right-to-Know" Program. IACM is committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing on the chemicals used by the color industry in order to assure their human and environmental safety. The category analysis, test plan, and robust summaries represent the first phase of IACM's commitment to the Chemical "Right-to-Know" Program.

2.2 BACKGROUND INFORMATION

This test plan provides data for FD&C Yellow 6 (Sunset Yellow). FD&C Yellow No. 6 is a yellow powder that is freely soluble in water and is used as a food colorant in diary products, snack foods, cereals, bakery items, confectionery products, frozen deserts and beverages, cosmetics, ingested and externally applied drugs, and dietary supplements.

FD&C Yellow No. 6 is an azo dye. Azo compounds are formed from arenediazonium ions reacting with highly reactive aromatic compounds, in what is called a diazo coupling reaction. Azo compounds are generally deeply colored because the azo linkage brings the two aromatic rings into conjugation [Solomon, 1996]. In addition to possessing extended conjugation, many azo dyes are also ring substituted with sulfonic acid substituents, which significantly increase polarity and water solubility and decrease absorption *in vivo*.

2.3 REGULATORY STATUS

FD&C Yellow 6 is a certified color additive approved in the United States to color food, drugs and cosmetics. Certified color additives are synthetic organic compounds that must meet high purity specifications established by the Food and Drug Administration (FDA) (see Table 1 below). Each batch of manufactured certified color in the United States is

tested by the FDA for compliance with these specifications [Frick and Meggos, 1988]. Certified color additives are among the most thoroughly studied of all food ingredients because of the rigorous testing for human health endpoints required by the 1960 Color Additive Amendments to the FD&C Act [Hallagan, 1991]. There are currently only seven certified color additives approved for food, drug and cosmetic use in the United States.

Table 1. US FDA Specifications

FD&C Yellow No. 6 shall conform to the following specifications and shall be free from impurities other than those named to the extent that such other impurities may be avoided by good manufacturing practice (21 CFR 74.706)

- Sum of volatile matter at 135°C (275°F) and chlorides and sulfates (calculated as sodium salts), not more than 13 percent.
- Water-insoluble matter, not more than 0.2 percent.
- Sodium salt of 4-aminobenzenesulfonic acid, not more than 0.2 percent.
- Sodium salt of 6-hydroxy-2-naphthalenesulfonic acid, not more than 0.3 percent.
- Disodium salt of 4,4'-(1-triazene-1,3-diyl)bis[benzenesulfonic acid], not more than 0.1 percent.
- Sum of the sodium salt of 6-hydroxy-5-(phenylazo)-2-naphthalenesulfonic acid, not more than 1 percent.
- Sum of the trisodium salt of 3-hydroxy-4-[(4-sulfophenyl)azo]-,7-naphthalenedisulfonic acid and other higher sulfonated subsidiaries, not more than 5 percent.
- 4-Aminoazobenzene, not more than 50 parts per billion.
- 4-Aminophenyl, not more than 15 parts per billion.
- Aniline, not more than 250 parts per billion
- Azobenzene, not more than 200 parts per billion.
- Benzidine, not more than 1 part per billion.
- 1,3-Diphenyltriazene, not more than 40 parts per billion.
- 1-(Phenylazo)-2-naphthalenol, not more than 10 parts per million.
- Lead (as Pb), not more than 10 parts per million.
- Arsenic (as As), not more than 3 parts per million.
- Mercury (as Hg), not more than 1 part per million.
- Total color, not less than 87 percent.

FD&C Yellow No. 6 was first listed for food use in the United States in 1929. In 1994, 994,406 kg of FD&C Yellow No. 6 dye and 283,680 kg of FD&C Yellow No. 6 lake were certified for use in the United States.

The World Health Organization/Food and Agriculture Organization Joint Expert Committee for the Evaluation of Food Additives (WHO/FAO JECFA) have also evaluated the safety of FD&C Yellow No. 6 used as a coloring agent in food. An average daily intake (ADI) of 0-2.5 mg/kg bw/day was assigned by JECFA in 1982 based on the extensive human toxicological information available (see Table 2 below).

Table 2. Regulatory Approvals/Consumption Limits[1]

USA	FD&C Yellow No. 6 may be safely used for coloring foods (including dietary supplements) generally in amounts consistent with good manufacturing practice, except that it may not be used to color foods for which standards of identify have been promulgated under section 401 of the act unless added color is authorized by such standards (21 CFR 74.706).
EEC	0-2.5 mg/kg (14th series, 1983)
JECFA	0-2.5 mg/kg (26th report, 1982)

Based on the long history of use of FD&C Yellow No. 6 in food, the many hazard assessments performed by the United States FDA and WHO/FAO JECFA, and the current regulatory status of FD&C Yellow No. 6, there is no compelling evidence that this substance should be further tested for human health endpoints in the EPA Chemical "Right to Know" Program.

2.4 STRUCTURAL CLASSIFICATION

FD&C Yellow No. 6 is principally the disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid. The trisodium salt of 3-hydroxy-4[(4-sulfophenyl)azo]-2,7-naphthalenesulfonic acid may be added in smaller amounts (USFDA-21 CFR 74.706). The diazo nucleus (-N=N-) contains a benzene ring substituted with a *p*-sulfonic acid group and a naphthalene ring substituted with *o*-hydroxy and *p*'-sulfonic acid groups.

[1] IACM, 2003

2.5 INDUSTRIAL PRODUCTION

FD&C Yellow No. 6 is manufactured by coupling diazotized sulfanilic acid with 2-napthol-6-sulfonic acid. The dye is isolated as the sodium salt and dried.

2.6 PHARMACOKINETICS AND METABOLISM

The major route of metabolism for FD&C Yellow No. 6 is bacterial azo reduction in the gut. The major metabolites of FD&C Yellow No. 6 are sulfanilic acid and amino-2-naphthol-6-sulfonic acid [Honohan *et al*., 1977].

Rats orally administered a single oral dose of 100 mg FD&C Yellow No. 6 (Sunset Yellow) excreted 0.8% of the intact dye in the feces [Radomski & Mellnger, 1962]. (^{14}C) Sunset yellow (labeled on C-8 of naphthalenic moiety) was orally administered to female rats, and urine and bile were collected. After 96 hours, 8.5% of the 1-amino-2-naphthol-6-sulfonic acid equivalent, 37.4% of the sulfonic acid equivalent and 0.3% of intact dye were excreted in the urine; biliary excretion of sunset yellow was 1.5% [Honohan *et al*., 1976]. In another study, female Simonsen/Sprague-Dawley rats orally administered 1 ml of an aqueous solution containing 2-25 mg of Sunset Yellow excreted 0.3 and 1.5% of the intact dye in the urine and bile, respectively, and 37% of the sulphanilic acid equivalents in the urine. More than 90% of the dye was excreted in the feces [Honohan *et al*., 1977].

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

FD&C Yellow No. 6 is a solid and decomposed without melting when heated to 390 °C [NTP, 1981]. Accordingly, the melting point of FD&C Yellow No. 6 was calculated to be 350 °C using modeling software [MPBPVPWIN EPI Suite, 2000].

3.1.2 Boiling Point

The boiling point of FD&C Yellow No. 6 was calculated to be 837 °C [MPBPVPWIN EPI Suite, 2000]. Technically, data for this endpoint are not required given that this material is a solid and would likely decompose upon heating to elevated temperatures.

3.1.3 Vapor Pressure

The calculated vapor pressure for FD&C Yellow No. 6 has been reported to be 1.43 X 10-22 mm Hg at 25°C [MPBPVPWIN EPI Suite, 2000]. Given the high molecular mass of FD&C Yellow No. 6 (452.37) and the estimated Henry's law constant for azo dyes of 10^{-15} atm-m^3/mol it is highly unlikely that FD&C Yellow No. 6 would exhibit any significant (less than 0.001 mm Hg) vapor pressure. This is predicted by the MPBPVPWIN model. Based on these data, the vapor pressure is less than 1 X 10^{-20} mm Hg.

3.1.4 Octanol/Water Partition Coefficients

Log K_{OW} value for FD&C Yellow No. 6 is –1.18 [KOWWIN EPI Suite, 2000]. The experimental log K_{OW} value would be difficult to obtain by OECD methods given the large difference between water solubility and anticipated solubility in octanol. Based on the observations that FD&C Yellow No. 6 is freely water soluble (190,000 mg/L) and

essentially insoluble in a relatively polar solvent like ethanol (10 mg/L) (Marmion, 1991, robust summary not included), it is anticipated that the log K_{OW} value for this substances would exceed 6.0.

3.1.5 Water Solubility

FD&C Yellow No. 6 has a reported water solubility of 190,000 mg/L at 2°C, 190,000 mg/L at 25 °C, and 200,000 mg/L at 60 °C [Marmion, 1991]. The solubility of FD&C Yellow No. 6 in 100% glycerol is 200,000 mg/L at 25 °C while the solubility in ethanol is reported to be 10 mg/L at 60 °C (Marmion, 1991, robust summary not included). The solubility of FD&C Yellow No. 6 in octanol is expected to be less than 1 mg/L.

3.1.6 New Testing Required

None.

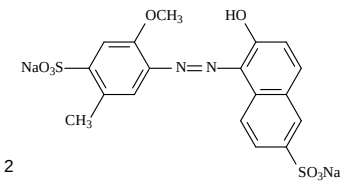
3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

Direct and indirect photolysis experiments were conducted on the structurally related monoazo dye, FD&C Red No. 40[2], using two 15-watt low pressure lamps as the ultraviolet light source. Following 50 minutes of exposure to the lamps, FD&C Red No. 40 concentration decreased by 7% in the direct experiment. In the indirect experiment which used acetone as the sensitizer, the concentration of FD&C Red No. 40 decreased by 99% after 20 minutes [Pasin and Rickbaugh, 1991]. The calculated half-life for FD&C Yellow No. 6 in hydroxyl radical reactions is 31.9 hours [AOPWIN EPI Suite, 2000].

3.2.2 Stability In Water

FD&C Yellow No. 6 does not contain functional groups (*e.g.*, esters, amides, acetals, epoxides, lactones, *etc*.) that hydrolyze in water. The only potential reactivity in water would involve desulfonation of the aromatic sulfonic acid or its corresponding sulfonic acid salt. In aqueous acid (sulfuric acid), aromatic sulfonic acids desulfonate at temperatures of 100 to 175 ^{o}C. These conditions would not typically be encountered in the environment. Therefore, FD&C Yellow No. 6 and its corresponding salts are anticipated to be stable in water.



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3.2.3 Biodegradation

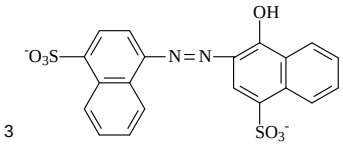
The biodegradability of azo dyes ring-substituted with a phenolic finction and two sulfonic acid groups consistently show that these substances are not absorbed onto activated sludge and, therefore, are not readily biodegradable [Shaul *et al*., 1990]. Incubation of 1.0 or 5.0 mg/L of a structurally related azo dye, (1-naphthalenesulfonic acid, 4-hydroxy-3-[(4-sulfo-1-naphthalenyl)azo]-, disodium salt)[3] with activated sludge from a sewage treatment plant revealed that the concentration of dye remained essentially constant in the influent flow, primary effluent, and activated sludge effluent. Essentially no azo dye was absorbed by activated sludge. Two other azo dyes ring-substituted with sulfonic acid groups (Acid Orange No. 10 and Acid Red No. 1) exhibited a similar behavior in these experiments. In an OECD 301C Guideline study D&C Red No. 9 (benzenesulfonic acid 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methyl, barium salt) was not readily biodegradable and was only 33% degraded after 21 days in a Zahn-Wellens test of inherent biodegradation (OECD SIDS 9th SIAM, 1999).

FD&C Yellow No. 6 was not predicted to be readily degradable by BIOWIN model calculations [BIOWIN EPI Suite, 2000].

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70 [EPIWIN EPI Suite, 2000]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log K_{OW}.

As expected, the model predicts that FD&C Yellow No. 6 is distributed completely to the water and soil compartments. Consistent with the extremely high water solubility and



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low log K_{OW} data, FD&C Yellow No. 6 showed no distribution to the fish compartment. These data are consistent with ecotoxicity data for aromatic sulfonic acid derivatives that demonstrate essentially no absorption and toxicity to fish even at concentrations exceeding 1000 mg/L.

3.2.5 New Testing Required

None.

3.3 ECOTOXICITY

Introduction

A broad range of azo dyes that contain naphthalenesulfonic acid or benzenesulfonic acid substituents exhibit a low order of toxicity in aquatic species. Reactive Black 5 (diazo) containing 4 sulfonic acid groups shows a very low toxic potential in aquatic organisms (fish LC_{50} 100-500 mg/l; bacteria EC50 > 2,000 mg/l) as well as the hydrolysed dye (fish LC_{50} > 500 mg/l; Daphnia magna EC50 (48h) > 128 mg/l) (Hunger & Jung, 1991, IUCLID). The inability of azo dyes to react with various groups of vital organic materials, such as proteins and DNA, reduces the potential hazard considerably (ETAD, 1991).

Spencer (1984) has examined the effect of Aquashade (a mixture of Acid Blue 9 and Acid Yellow 23*) on the oxygen consumption of the crayfish *Orconectes propinquus* and has not found any effect at a concentration of 1 mg/l at an exposure of five days.

A survey of available fish toxicity data on over 3,000 commercially available organic dyes by ETAD member companies indicated that about 98% have a LC50 greater than 1 mg/l, a concentration at which coloring of a river normally would be observable. Dyes containing more than one sulfonic acid group show LC50 values > 100 mg/l.

No adverse effects on the carp (*Cyprinus carpio*) exposed to less than 10 mg/l of 30 water soluble (ionic) and 12 disperse dyes for 8 weeks (Brown, 1987). The table below provides a general overview of the testing results for a broad range of dyes including acid dyes and mordant dyes which contain sulfonic acid substituents

Zebra fish is susceptible to (in declining order) basic dyes > acid dyes > disperse dyes at a level less than 100 mg/l with acid dyes at >10 mg/l. For the other chemical classes, hydrolysed reactive, direct and mordant dyes with sulfonic acid groups, the LC50 is above 100 mg/l. The susceptibility to acid and basic dyes for fish is in agreement with the other findings (Clarke and Anliker, 1980).

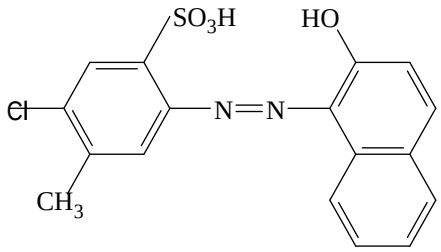
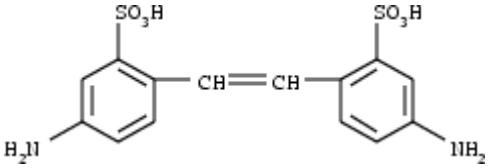
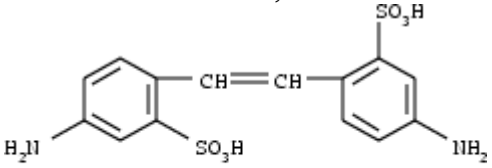
The susceptibility of Daphnia resembles that of the zebra fish, but the order is different, basic > disperse > acid. The remaining chemical classes all show a LC50 above 100 mg/l. The study confirms the findings reported by other researchers (Hunger and Jung, 1991; IUCLID) that the reactive dyes and hydrolysed reactive dyes have a low toxic potential in aquatic organisms. One fish species (*Oryzias latipes*), exposed 48 hours to Acid Yellow 36, had a LC_{50} of 68 mg/l. For the remaining dyes, amongst them 5 acid, 6 direct and 2 solvent dyes, the LC50 was above and well above 100 mg/l. Apparently, the different fish species show very variable susceptibility.

3.3.1 Acute Toxicity to Fish

Based on input parameters for molecular weight (452.37), water solubility (190,000 mg/L at 25 °C), and melting point (390 °C), the calculated 96-hour LC50 for FD&C Yellow No. 6 is 6,044 mg/L [ECOSAR EPI Suite, 2000] indicating a very low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 6 is to a large extent, a function of the presence of aromatic sulfonic acid and phenolic ring substituents. The presence of more than one aromatic sulfonic acid group enhances water solubility and decreases absorption by aquatic species. Two structurally related substituted azo colorants containing naphthalene sulfonic acid and benzene sulfonic acid residues have been the subject of ecotoxicity studies in fish. Both exhibit a very low order of acute toxicity. The structural relative barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid has been studied in two fish species (*Brachydanio rerio* and *Oryzias latipes*). The 96 hr- LC50 exceeded 500 mg/L, one in a semi-static test and the other in a static test (Hoechst AG, 1992). In other acute fish toxicity tests, the structurally related azo dye, 2-naphthalenecarboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt showed an 96-hr LC50=33 mg/L in Orange killifish (MITI, Japan, 1992). The fact that FD&C Yellow No. 6 contains two sulfonic acid groups and one phenolic group while structural analogs contain only one sulfonic acid group and one carboxylic acid group supports the conclusion that Yellow No. 6 is anticipated to exhibit even a lower toxicity than do the listed analogs. Both model data and data on these analogs support the conclusion that Yellow No. 6 exhibits a very low order (LC50 >100 mg/L) of toxicity for fish.

The presence of sulfonic acid residues in similar aromatic compounds limits absorption and concomitant toxicity in aquatic species. The extensive studies on the ecotoxicity of aromatic sulfonic acids indicate a very low order of toxicity to fish [Greim *et al.*, 1994]. Experimental LC50 values are available for stilbene sulfonic acids in which the N atom in the diazo dye is replaced by C. As indicated in Table 3 below, acute fish toxicity studies on salts of stilbene sulfonic acid derivatives result in a 96-hour LC50 value greater than 10,000 mg/L. Also, 48-hour and 72-hour LC50 concentrations of 200 and greater than 1000 mg/L, respectively have been reported [Greim *et al.*, 1994]. These values are consistent with calculated values.

Table 3

Name	Acute Toxicity to fish
barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid 	96-hour LC50: >500 mg/L in *Brachydanio rerio* 96-hour LC50: >420 mg/L in *Oryzias latipes*
2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid 	48-hour LC50: 200 mg/L
2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt 	72-hour LC50: greater than 1000 mg/L

• 2 Na

Given the high-calculated LC50 values from the ECOSAR model and the experimentally measured toxicity of azo dyes containing aromatic sulfonic acid substituents, no additional testing is requested.

3.3.2 Acute Toxicity to Aquatic Invertebrates

The calculated 48-hour LC50 value for FD&C Yellow No. 6 in *daphnids* is 486.5 mg/L based on input parameters for molecular weight (452.37), water solubility (190,000 mg/L at 25 °C), and melting point (390 °C), [ECOSAR EPI Suite, 2000] indicating a low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 6 is to a large extent, a function of the presence of aromatic sulfonic acid phenolic ring substituents. This physiochemical property limits absorption and subsequent toxicity. Acute toxicity data on structurally related azo dyes containing sulfonic acid and hydroxy constituents support this conclusion.

Experimental data for the two azo colorants containing a benzene sulfonic acid or naphthalene sulfonic acid and a carboxylic acid substituents (barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid and 2-naphthalene carboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt) show low levels of toxicity in *Daphia magna.* In one study, an OECD 202 guideline study, the EC50 is reported to be 280 mg/L (EA, Japan, 1992). The extensive studies on the ecotoxicity of aromatic sulfonic acids also indicate a very low order of toxicity to aquatic invertebrates [Greim *et al.*, 1994]. An experimental 24-hour EC50 value with *Daphnia* for a stilbene sulfonic acid derivative, 2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, was greater than 100 mg/L [Greim *et al.*, 1994]. The fact that calculated values for FD&C Yellow No. 6 agree with experimental values for azo dyes containing sulfonic acid and phenolic constituents and aromatic sulfonic acid derivatives, compounds that have limited absorption, supports the conclusion that FD&C Yellow No. 6 exhibits a low order of toxicity to aquatic invertebrates (>100 mg/l).

The extensive studies on the ecotoxicity of aromatic sulfonic acids indicate a very low order of toxicity to aquatic invertebrates [Greim *et al.*, 1994]. An experimental 24-hour EC50 value with *Daphnia* for a stilbene sulfonic acid derivative, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, was greater than 100 mg/L [Greim *et al.*, 1994]. This value is consistent with calculated values.

3.3.3 Acute Toxicity to Aquatic Plants

Based on input parameters for molecular weight (452.37), water solubility (190,000 mg/L at 25 °C), and melting point (390 °C), the calculated 96-hour EC50 for FD&C Yellow No. 6 with green algae is 146,000 mg/L [ECOSAR EPI Suite, 2000] indicating a very low order of acute toxicity. In a 96-hour algal chronic toxicity test, a sulfonic acid substituted azo dye, stimulated population growth (26.4%) compared to control (algal assay medium) [Greene and Baughman, 1996]. In fact, of the 46 dyes tested, only one, an anthraquinone dye, produced measurable toxicity. Given the high-predicted value for acute toxicity to aquatic plants and the stimulation of plant growth resulting from the addition of a structurally related azo dye in an experimental acute toxicity test, it is not recommended that additional tests be performed.

3.3.4 New Testing Required

None.

[HUMAN HEALTH TOXICITY

3.3.5 Acute Toxicity

The low acute oral toxicity of FD&C Yellow No. 6 is reflected by LD50 values greater than 2,000 mg/kg [Lu and Lavalle, 1964] and 10,000 mg/kg [Gaunt *et al*., 1967] in rats, and greater than 6,000 mg/kg in mice [Gaunt *et al*., 1967].

In a pre-GLP acute toxicity study, adult male Wistar rats were administered 2000 mg/kg bw of FD&C Yellow No. 6 *via* stomach tube. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 2000 mg/kg bw [Lu and Lavalle, 1964].

In another pre-GLP acute toxicity study, groups of five male and female rats each were administered the FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. No deaths at up to 10,000 mg/kg bw. Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 2000 mg/kg bw [Gaunt *et al*., 1967].

Groups of five male and female mice each (body weights: 20-25 kg) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. No deaths at up to 6000 mg/kg bw Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 in mice was determined to be greater than 6000 mg/kg bw [Gaunt *et al*., 1967].

Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. Slight diarrhea reported for 24

hours following treatment. Skin, feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 3800 mg/kg bw [Gaunt *et al*., 1967].

Groups of five male and female mice each (body weights: 20-25 kg) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 5500 mg/kg bw [Gaunt *et al.*, 1967].

3.3.6 *In vitro* and *In vivo* Genotoxicity

3.3.6.1 In vitro

FD&C Yellow No. 6 tested negative in reverse mutation assay using TA1535, TA1537, TA98, TA100; TA92 and TA94 with and without metabolic activation [Chung *et al*., 1981; Ishidate *et al*., 1984; Muzzall and Cook, 1979]. In one chromosomal aberration test, FD&C Yellow No. 6 tested positive at concentrations up to 6,000 micrograms/mL without metabolic activation [Ishidate *et al*., 1984], but tested negative in another chromosomal aberration test at a concentration up to 5,000 micrograms/mL with and without metabolic activation [Ivett *et al.*, 1989]. FD&C Yellow No. 6 gave a response judged to be equivocal in the sister chromatid exchange assay (SCE) at concentrations up to 5,000 micrograms/ml [Ivett *et al*., 1989].

3.3.6.2 In vivo

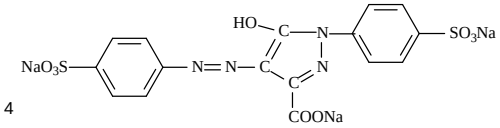
In a rodent micronucleus test, 10 ml/kg bw male rats were administered a single oral dose of 500 or 1000 mg/kg of FD&C Yellow No. 6. Bone marrow samples were taken at 24 and 48 hours later. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point in either species. There was also no reported increase polychromatic erthyrocytes [Westmoreland and Gatehouse, 1991].

In an in vivo UDS assay, six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg/kg bw of the structurally related dye FD&C Yellow No. 5[4] *via* gavage. FD&C Yellow No. 5 did not induce unscheduled DNA synthesis at the dose level tested [Kornbrust and Barfknecht, 1985].

3.3.7 Repeat Dose Toxicity

Groups of ten male and ten female mice each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet *ad libitum.* The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Mean body weight gain was decreased compared to controls among male mice receiving the 100,000 ppm intake level. Decreases in body weight gain were also reported for female mice at all intake levels, and was dose related from 12,500 ppm to 100,000 ppm. Gross and histopathological examinations revealed no treatment related lesions in male or female mice at any intake level. The NOAEL's were reported to be 50,000 ppm and less than 6,000 ppm for male and female mice, respectively [NTP, 1981].

Groups of ten male and ten female rats each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet *ad libitum.* The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. No animals died during the study. Decreases in mean body weight gain were reported for male rats at the 25,000, 50,000 or 100,000 ppm intake levels. For female rats, decreases in mean body weight gain were reported at the 12,500, 25,000, 50,000 or 100,000 ppm intake levels. Bone marrow hyperplasia was reported in all examined



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animals at the 50,000 or 100,000 ppm intake levels. The NOAEL's were reported to be 6000 ppm for female rats and 12,500 ppm for male rats [NTP, 1981].

Groups of fifty male and fifty female mice each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Fifty male and female mice each served as concurrent controls. Animals were housed five per cage and fed the test diet *ad libitum*. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. The mean body weights of male and female mice administered the high dose were slightly lower than the control animals throughout most of the study. The survival of male and female mice was similar between treated animals and controls (males: control 38/50 (76%); low dose 40/50 (80%); and high dose 33/50 (66%) and females: control 38/50 (76%); low dose 35/50 (70%) and high dose 43/50 (86%)). An increased incidence in hepatocellular carcinomas was reported among males in the low (46%) and high (32%) dose groups compared to the control males (26%), but was only a significant difference in the low dose mice. No significant differences were observed in the female animals. The increased incidence in hepatocellular carcinomas reported for male mice was not considered clearly related to administration of the test material given the variability in tumor occurrence in control male B6C3F1 mice and because the incidence of these tumors was not significantly increased in the high dose male mice. The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD&C Yellow No. 6 in B6C3F1 mice [NTP, 1981].

Groups of fifty male and fifty female rats each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Ninety male and female rats each served as concurrent controls. Animals were housed five per cage and fed the test diet *ad libitum*. The animals were observed twice per day and weighed at least monthly.

Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included the adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. The mean body weights of male rats administered the high dose were slightly lower than the control animals throughout the study. The survival of male and female rats was similar between treated animals and controls (males: control 70/90 (78%); low dose 36/50 (72%); and high dose 38/50 (76%) and females: control 66/88 (75%); low dose 40/50 (80%) and high dose 37/50 (74%)). Histopathological examination revealed no evidence of carcinogenicity related to treatment with the test material. No other effects were reported. The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD&C Yellow No. 6 in F344/N rats [NTP, 1981].

3.3.8 Developmental Toxicity

FD&C Yellow No. 6 was administered to 140 Charles River CD rats by gavage at dose levels of 100, 300, or 1000 mg/kg bw/day. Three negative control groups (20/group) received 0.5% methocel and one positive control group (20 rats) received 7.5% retinoic acid. All females were dosed on days 6-15 of gestation. No teratogenic effects were observed in the offspring of rats receiving up to 1000 mg/kg bw/day [International Research and Development Corporation, 1972]. Also, there were to evidence of teratogenicity in the offspring of rats maintained on diets containing 0.75, 1.5, or 3.0 % Yellow No. 5 two months prior to and during mating. Females were then maintained on these diets during gestation and post-weanling. The offspring of this study were also maintained on these diets for their lifetime (approximately two years). No evidence of carcinogenic, genotoxic, or teratogenic effects was noted in this study (Bio-dynamics, 1981) or in a subsequent study at a 5.0% dietary level (Bio-dynamics, 1982).

3.3.9 Reproductive Toxicity

Two long-term *in utero* studies have been performed in rats. In the first study, groups of male and female rats were maintained on diets containing 0, 0.75, 1.5, or 3.0% of Yellow 6 for approximately two months. Single males and females were housed together in a 1:1 ratio for a one week mating period. After gestation and a 21-day lactation period, pups were weaned and remained together for 13 to 19 days until selection of F1 animals.
Animals were housed individually in elevated stainless steel cages, except during mating, lactation and post-weaning phases. Water was provided ad libitum by an automated water system. Animals were maintained on a 12-hour light/dark cycle. Bodyweights, food consumption, hematology and clinical chemistry parameters, absolute and relative organ weights, survivorship, and tumor incidence data were statistically analyzed.

For the Fo generation general appearance, behavior, survival were monitored twice daily. Detailed physical examination was performed weekly and ophthalmoscopic examination was performed pretest. Male body weights were recorded twice pretest and weekly during the premating and mating periods. Female body weights were recorded twice pretest and weekly during the premating, mating, and gestation periods and on days 0, 4, 14, and 21 of lactation. Male food consumption was measured pretest, and weekly during the premating period and female food consumption was measured pretest, weekly during the premating period and for the first two weeks of gestation.

Pregnant females that were sacrificed post-weaning were not subjected to necropsy. Animals dying spontaneously or killed in moribund condition were given a complete gross postmortem examination. For pups viability and mean body weights were recorded at days 0, 4, 14, and 21. For F1 generation general appearance, behavior, and survival were monitored twice daily. Detailed physical examination were performed weekly and ophthalmoscopic examination were performed initial and at months 3, 6, 12, 18, and 24
Body weights were recorded post weanling and then weekly through 13 weeks, biweekly 14 through 26 weeks, approximately monthly thereafter and terminally (after fasting). Individual and mean body weights were furnished at weeks -1 (initial weights following

random selection), 1, 4, 8, 13, 26, 51, 76 and 100 for both sexes and at week 120 for females and at week 128 for males.

Food consumption data were obtained and furnished for same intervals as body weights. Hematology, clinical chemistry and urine analysis were performed on 10 rats/sex/group at months 3, 6, 12, 18 and 24. Hematology included measurements of heamoglobin, hematocrit, erythrocyte counts, total and differential leukocyte counts, and erythrocyte morphology. Clinical chemistry included measurement of serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, blood urea nitrogen, fasting blood glucose, total protein, and creatinine while urine analysis measured gross appearance, specific gravity, pH, protein, glucose, ketches, bilirubin, occult blood, and microscopic analysis.

Animals dying spontaneously or killed in moribund condition were given a complete gross postmortem examination. Necropsy was performed on 10 rats/sex/group sacrificed at month 12 and on all survivors at termination of the study. Animals were sacrificed by ex-sanguination under ether anesthesia. Individual and mean terminal body weights and absolute and relative weights of brain, gonads, thyroids, spleen, kidneys, and liver of I0 rats/sex/group sacrificed at month 12 and of all antis sacrificed at termination of study Histopathological examination was performed on tissues from all organs weighed plus about 28 other tissues were examined microscopically from i0 rats/sex/group from each control group and from the high dietary level group at the interim sacrifice and from all survivors from these groups at termination as well as of any animal dying spontaneously or sacrificed in extremis from these groups. In addition, microscopic examination of tissues exhibiting gross changes of uncertain nature and of all tissue masses was performed for all animals.

The major adverse findings in the study occurred at the 3.0% level. For the female rats on the 3.0% dietary level of FD&C Yellow No. 6 compared to the pooled control groups of female rats sacrificed at termination of the study, both mean absolute and relative kidney weights were increased. Only the increase in mean relative weight of the kidneys was

reported to be statistically significant. The increase in absolute kidney weights in spite of the decrease in mean body weight might indicate the kidneys were enlarged in this test group.

Histopathological examination of the rats through termination of the study revealed increased incidences of female rats with adrenal medullary adenoma (13/69 or 18.8%) on the 3.0% dietary level compared to the incidence of control females with the lesions (10/139 or 7.2%). Because of the increased incidence of the adrenal lesions seen in this study in the 3.0% dietary level females and in the 5.0% dietary level females of the high dose study NTP study, the test laboratory re-sectioned the adrenals and reexamined the adrenal microslides of females in the two studies. These slides were also examined by FDA/CFSAN pathologists. On the basis of the pathologist's findings and other considerations, the Cancer Assessment Committee concluded that the increases in the number of female rats with adrenal medullary lesions are unrelated to treatment with FD&C Yellow No. 6 (FDA, December 3, 1985). In a second long term in utero study using the same study protocol, groups of rats were maintained on diets of 0 and 5.0 % Yellow No. 6 for a similar period of time for the Fo and F1 generations (Biodynamics, 1992)

Histopathological examination also revealed an increased incidence of rats with testicular interstitial cell adenoma in the group on the 3.0% dietary level (15/70 or 21.4%) compared to the incidence (14/138 or 10.1%) in the pooled control groups. The incidence for the treated group is near the maximum control incidence reported by Bio/dynamics Inc., in this rat strain. This was in the study of FD&C Blue No. 2 where the reported incidence of testicular interstitial cell adenoma was 27/137 or 19.7% for the contemporary pooled control groups. It was concluded that the increased incidence of rat testicular interstitial ceil adenomas was not treatment-related because the rats on the 5.0% dietary level in the NTP chronic study did not show an increased incidence. The incidences of testicular tumors were concluded to be unrelated to administration of the test vehicle.

In a three-generation reproduction study, 150 Charles River CD rats (10 males and 20 females/group/generation) received FD&C Yellow No. 6 at dietary levels of 0, 5, 50, 150, or 500 mg/kg/day. Rats treated with a positive control showed increased number of abnormal live pups and a concomitant decrease in live normal pups. No such changes occurred in the treated groups of animals. The mean weights of the offspring from the 300 and 1000 mg/kg bw/day groups were decreased when compared to the mean fetal weight of one of the three negative controls. However, there difference in mean body weight of the 300 and 1000 mg/kg bw/d groups was not statistically different from the combined negative control group mean or the two other individual control group means. Therefore, it was concluded that there was no significant difference in mean fetal weights between test and control animals. There were no compound related effects on early or late resorptions, empty implantation sites, body weight or numbers of live or dead fetuses. There was a statistical increase in the number of abnormal young in the positive control group treated with 7.5 mg/kg bw/d of retinoic acid. No treatment-related effects were observed in the parental rats or the pups receiving oral doses of up to 500 mg/kg bw/day [International Research and Development Corporation, 1974].

Based on the results of the 3-generation study and two long term *in utero* studies in which pretreated male and female rats were mated, females were treated through gestation and weaning, and pups were treated througout their lifetime, there is no evidence that Sunset Yellow exhibits any potential for reproductive toxicity

New Testing Required

None.

3.4 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	**Boiling Point**	**Vapor Pressure**	**Partition Coefficient**	**Water Solubility**
Sunset Yellow **CAS No. 2783-94-0**	A	Calc	Calc	Calc	A

Chemical	Environmental Fate and Pathways			
	Photodegradation	**Stability in Water**	**Biodegradation**	**Fugacity**
Sunset Yellow **CAS No. 2783-94-0**	R, Calc	NA	R, Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	**Acute Toxicity to Aquatic Invertebrates**	**Acute Toxicity to Aquatic Plants**
Sunset Yellow **CAS No. 2783-94-0**	R, Calc	R, Calc	R, Calc

Chemical	Human Health Data					
	Acute Toxicity	**Genetic Toxicity *In Vitro***	**Genetic Toxicity *In Vivo***	**Repeat Dose Toxicity**	**Repro-ductive Toxicity**	**Develop-mental Toxicity**
Sunset Yellow **CAS No. 2783-94-0**	A	A	A	A	A	A

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

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